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RESEARCH ARTICLE

ASSESSMENT OF HEAVY METALS IN WATER AND SEDIMENTS BY ICP-AES IN COASTAL AREA OF CHIRACKAL, ERNAKULAM DISTRICT, KERALA

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ABSTRACT

The study was to examine the physico-chemical parameters and the concentration of heavy metals in water and sediment samples of Chirackal mangrove area, Ernakulam, Kerala. The parameters determined include pH, Alkalinity, Salinity, Chloride, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), % carbon and % organic matter by titrimetric method, phosphate, nitrite, nitrate and sulphate and silicate by UV-Visible Spectrophotometer as well as the concentration of heavy metals (Cd, Cr, Cu, Fe, Pb, Co, Ni, Mn and Zn) in water and sediment samples by Inductively Coupled Plasma-Atomic Emission Spectrophotometer (ICP-AES) after pretreatment of the samples with microwave digestion system.. The results indicated that the average concentration of the metals in water and sediment on the periphery (station 1) was more when compared to the other two stations. Mangrove sediments showed high Fe (2441mg/g), Cu (6.04mg/g), Cd (2.83mg/g), Zn (61.04mg/g), Mn (28.36mg/g) and Pb (2.66mg/g) concentrations than water samples. The highest average concentration for Pb in water was 1.81ppm in station 2 and for sediment was 2.11ppm in station 1. Also, the highest average concentration reported for Cr in water and sediment was 2.22 ppm and 2.36 ppm respectively. With regard to the results concentration of the metal, the area contain low Cd, Cr, Ni and Pb contents.

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INTRODUCTION

The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years, because they are indestructible and most of them have toxic effects on organisms (Mac Farlane and Burchett, 2000). Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bio accumulate in aquatic ecosystems (Censi *et al.,* 2006). Many of the water and sediments in our rivers, lakes, and oceans have been contaminated by pollutants. Some of these pollutants are directly discharged by industrial plants and municipal sewage treatment plants, others come from polluted runoff in urban and agricultural areas, and some are the result of historical contamination. The entry of municipal, industrial, and agricultural waste into the environment is another way of the environment pollution by human. As municipal, industrial, and agricultural waste enters the water, biological and chemical contaminants including heavy metals also enter water resources.

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Although some of these metals are essential as micronutrients, their high concentration in the food chain can cause toxicity and environmental impacts and endanger aquatic ecosystems and their users (Prabu, 2009; Kane et al., 2012). The surface water resource is very much essential, because of its human and animal living, aquatic flora and fauna, agriculture (Haque, 2008). Pollution of heavy metals in aquatic system is growing at an alarming rate and has become worldwide problem (Malik et al,2010). Heavy metals like Cu, Zn, Fe, Ni, Cd, Cr, Co, Pb etc. are usually present in water at low concentration, but enhanced concentration of these metals have found as a result of human activities(Mohiuddin et al., 2010; Akbal et al., 2011; Zakir et al., 2011;Shikazono et al., 2012). Sediments aggregates contaminants over time, and are in constant flux with the overlying water column (Bai et al., 2010; Deng et al., 2010; Ayejuyo et al., 2010; Usero et al., 2005; Martin and Whitefield, 1983). The analysis of metals in water and sediments permit detection of pollutants that may be either absent or in low concentration in the water column (Adeniyi et al., 2008; Yusuf and Osibanjo, 2006a). Heavy metals such as copper, iron, chromium and nickel are essential metals since their play an important role in biological systems, whereas cadmium and lead are non-essential metals, as they are toxic, even in trace amounts (Verardo et al., 1990; Fernandes *et al.,* 2008). The present study is intended to estimate the water quality parameters and the heavy metal concentration that deteriorate the purity of water and sediments as well as the growth of other organisms.

MATERIALS AND METHODS

Study area

Chirackal is situated between 9.927658^o N and 76.255159^o N. Chirackal mangrove area is affected by municipal and industrial pollution during recent days. Three sampling stations were selected on the basis of water quality parameters, human and environmental factors and the probability of anthropogenic pollution.

Chirackal

Sample Collection

Total 144 samples (72 water samples and 72 sediment samples) were collected from three locations of the lake every month (three samples of water and three samples of sediment) for 2 years. Pre-cleaned sampling bottles were immersed about 10 cm below the water surface in the periphery, 25 meters away from the periphery and from the centre of the lake. About 1L of the water samples were taken at each sampling site. Samples were acidified with 10% HNO3, filtered and kept at 4 °C until analysis. 300 g of sediment samples from the same locations were collected and stored in pre-cleaned polythene bags for processing.

Analytical Methods

On site measurement and laboratory analyses were carried out as per standard methods. On site measurement included fixation of dissolved oxygen (DO) and temperature. Dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH, alkalinity, total dissolved solids (TDS), total CO_2, ammoniacal - nitrogen (NH_3N), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), chloride, phosphate (PO_4^{3-}), sulphate (SO_4), calcium, magnesium, sodium and potassium were analyzed as per APHA Standard Methods (APHA, AWWA, WEF, 1995). For analysis of metals using Inductively Coupled Plasma Atomic Emission Spectrophotometer (Optima 4100 DV ICP-AES) (APHA, 1995). Detection limits of the instrument are given in Table 1. The detection limit is defined as the lowest analytical signal to be distinguished qualitatively at a specified confidence level from the background signal (Kackstaetter and Heinrichs, 1997). The accuracy of analytical procedure was checked by analyzing the standard reference materials (water: SRM-143d, National Institute of Standards and Technology; sediment: CRM-277, Community Bureau of Reference). Recovery rates ranged from 79 to 96% for all elements the investigated. Similar range the accumulation level for heavy metal concentrations is reported by Dang (2005).

Table 1. Spectral lines used in emission measurements and the instrumental detection limit for the elements measured by using ICP- AES

Element	Wave length(nm)	Instrumental Detection Limit
Cd	228	0.01
Cr	267	0.07
Cu	324	0.014
Fe	259	0.07
Ni	231	0.01
Pb	220	0.03
Co	280	0.01
Mn	264	0.02
Zn	270	0.02

RESULTS AND DISCUSSION

Physico-chemical Parameters

The result of physico-chemical analysis of water samples at an average of four months from three stations of Chirackal mangrove area is presented in Figure 1 and 2. Temperature and pH of water samples ranged from 26.42-29^{0}C and 6.88-7.61 and respectively. It was observed that the sample with highest alkalinity was 76 mg/l (station 2) and the lowest was 22 mg/l (station 3). The changes in alkalinity due to the discharge and precipitation from the river. Eddy *et al*.,(2004) reported that in the Cross River estuary, changes in river discharge significantly increases salinity. The high value of alkalinity at station 2 is due to the continuous intrusion of salt water. Eddy and Etuk (2004a) have suggested that changes in physico-chemical properties of a water body is due to tidal mixing of water. The salinity of the lake was found to correlate positively with most of the measured physicochemical parameters.

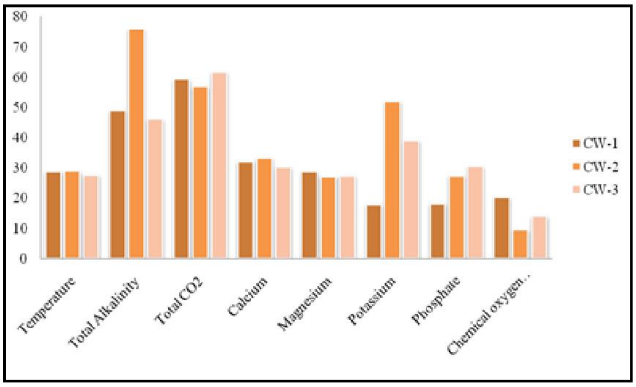

Figure 1. Showing the mineral concentration of water samples (1)

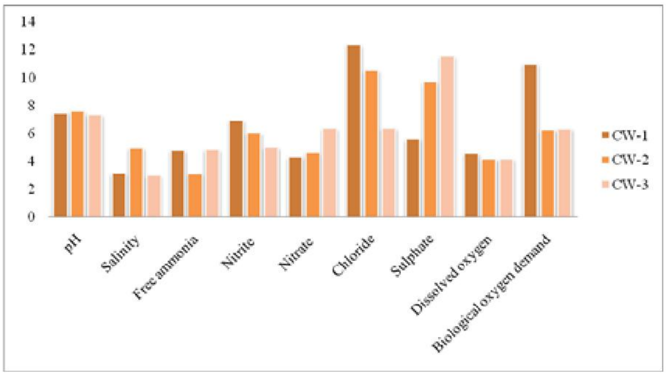

Figure 2. Showing the mineral concentration of water samples (2)

Alkalinity ranged from 37 mg/l to 76 mg/l. Total dissolved solids was reported high (3889.96 mg/l) in station 2 and low (2673.88) in station 1.Total CO_2 ranged from 51.58 mg/l to 61.83 mg/l. The water samples from the three sampling stations, Ca and Mg concentration differed slightly and were maximum in station 1(23.04 mg/l) and station 3 (25.91 mg/l) respectively. Both Na and K reported high in station 2 (191 mg/l and 52 mg/l). The nitrite and nitrate concentration in the water samples ranged from 2.41mg/l-6.94 mg/l and 3.66 mg/l-6.36 mg/l. The presence of nitrite in water bodies is due to the reduction of nitrate. The highest ammonium concentration (4.84 mg/l) was reported in station 3. Introduction of nitrogen containing waste into the lake and run-off water from farm lands where nitrogenous fertilizers had been applied are the reason for the high ammonia level (Ademoroti, 1996; Dara,2003). The levels of chloride ranged from 3.95 mg/l to 12.35 mg/l (station 1). The sulphate (11.52 mg/l) and phosphate (30.51 mg/l) concentration showed high in station 3. The dissolved oxygen ranged from 2.02 mg/l to 4.60 mg/l. The dissolved oxygen levels obtained in this study are similar to the values reported for Calabar River estuary (Eddy and Etuk, 2004b) and higher the values reported for River Ethiope (Egboh and Emeshili, 2007). Adequate DO is necessary for good water quality, survival of aquatic organism and decomposition of waste by microorganism (Islam et al., 2010; Rahman et al., 2012). Akkoyunlu and Akiner (2012), Rosli et al (2012) have measured the water qualitative parameters such as BOD and DO to indicate the relationship between the level of pollution and the water quality. The result of the mineral analysis of sediment samples is shown in Figure 3 and 4.

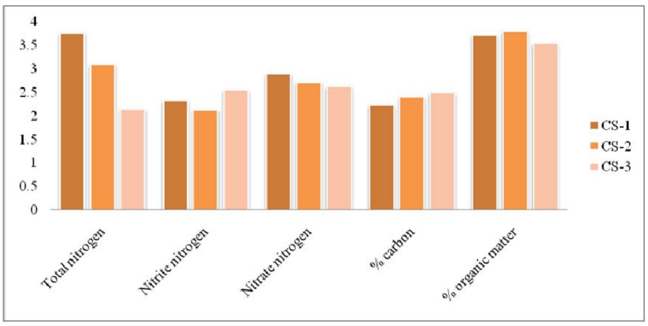

Figure 3. Showing the mineral concentration of Sediment samples (1)

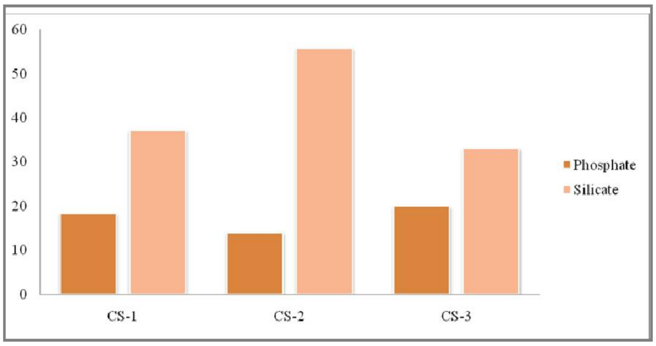

Figure 4. Showing the mineral concentration of Sediment samples (2)

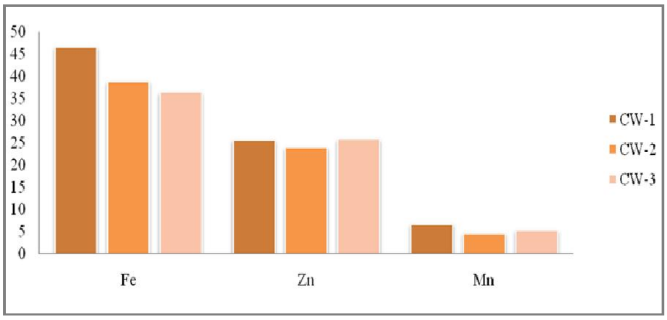

Figure 5. Showing the metal concentration in water samples (1)

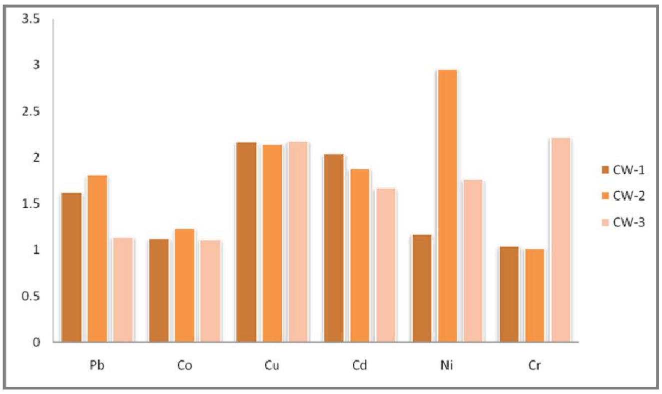

Figure 6. Showing the metal concentration in water samples (2)

In case of sediments, high concentration of phosphate was reported in station 1 (28.50 mg/l) and low in station 3 (13.73 mg/l). Silicate concentration ranged from 55.80 mg/l to 137.10 mg/l, ie, CS 3> CS 1> CS 2. Total nitrogen was reported high in station 1 (3.77 mg/l) and low in station 3 (1.71 mg/l). Nitrite and nitrate content in sediment samples were reported maximum in station 3 (1.95 mg/l and 2.89 mg/l) and minimum in station 1 (1.42 mg/l and 1.84 mg/l). % carbon and % organic matter ranged from 1.88 mg/l (station 2) to 2.49 mg/l(station 3) and 1.97 mg/l (station 1) to 3.80 mg/l(station 2) respectively. Yusuf and Osibanjo (2006b) stated that physicochemical characteristics of water affect the precipitation of these elements in sediment. The results of the metal analysis of water and sediment samples in three stations of Chirackal revealed that the mean concentration of Cd, Co, Ni, Cr, Cu, Fe Mn, Pb, and Zn in the bank of the lake was higher than that of water from the centre. Metal concentrations in sediment increase with the decrease of the particle size and increase of organic matter content (Halcrow *et al*., 2007).The heavy metal concentrations in water and sediment samples in the three stations were compared with international standard (WHO,1993; WPCL, 2004). Heavy metal concentration in water samples (mg/l) is shown in Figure 5,6 and sediment samples (mg/kg) in Figure 7,8.

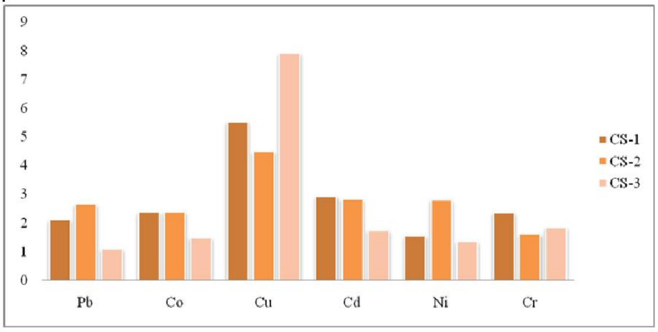

Figure 7. Showing the metal concentration in sediment samples (1)

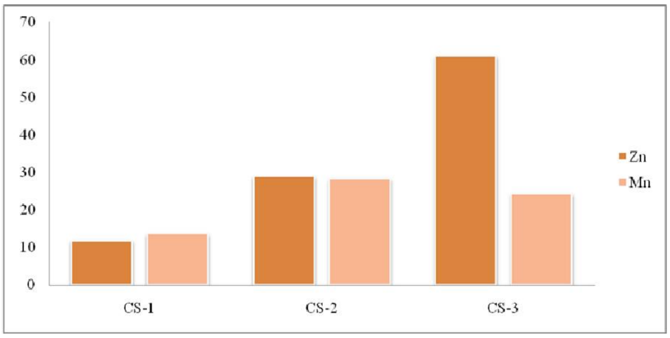

Figure 8. Showing the metal concentration in sediment Samples (2)

The above results showed that, mangrove sediments showed high Fe (2441 mg/l), Cu (6.04 mg/l), Cd (2.83 mg/l), Zn (61.04 mg/l), Mn (28.36 mg/l) and Pb (2.66 mg/l) concentrations than water samples. Maximum Fe in sediment sample (2441 mg/l) was reported at station 3 and 46.74 mg/l for water sample, ranged from 36.47 mg/l to 46.74 mg/l. In both sediment and water samples, the Cd concentration was reported high in station 1 (2.04 mg/l and 2.94 mg/l) of the sampling area. Cd ranged from 0.99 mg/l (station 2) to 2.04 mg/l (station 1) in water and 1.16 mg/l (station 3) to 2.94 mg/l (station 1) in sediment samples. A high-concentration range of 0.01 to 0.26 mg/l Cd has been reported in the Umtata River (Fatoki et al., 2002). A higher Cd concentration range of between 0.008 ± 0.003 and 0.017 ± 0.002 mg/l in Tyume River has been previously reported (Fatoki and Awofolu, 2003). Compared to other stations, station 3 is reported with maximum Cr content in water (2.22 mg/l) and station 1 with maximum Cr in sediment (2.36 mg/l). Station 1 showed minimum Cr (1.04 mg/l) level in water. Apart from natural sources, other probable sources of this metal in water include leaching from Ni-Cd based batteries (Hutton et al., 1987; Binning and Baird, 2001), runoff from agricultural soils where phosphate fertilizers are used (Stoeppler, 1991) and other metal wastes. High Cu content was reported in the sediment samples of station 3 (7.90 mg/l) and station 1 showed minimum (2.18 mg/l). In case of water, station 1 showed maximum (2.30 mg/l) and station 2 showed minimum (2.09 mg/l) Cu concentration. Station 2 showed high Ni concentration in both water (2.96 mg/l) and sediment (2.01 mg/l) samples. High Co content was reported in sediment (2.14 mg/l) in station 1 and less in station 3 (0.65 mg/l). In water, Co ranged from 0.68 mg/l (station 1) to 1.14 mg/l (station 2). Cobalt is regarded as an essential element and forms part of Vitamin B12 required for red-blood cell synthesis. Elemental Co is not found in nature but exists variously as sulphide ores and in association with As, Fe, Ni and Cu. Other possible sources in the river water include wastes from some metal alloys and cobalt salts in some pigments. The range of Zn content in sediment was 8.74 mg/l (station 1) to 60.87 mg/l (station 2)and water was 11.94 mg/l (station 2) to 25.91 mg/l (station 2). The TWQR levels of Zn in water for safe aquatic ecosystem (DWAF, 1996b), irrigation and livestock watering (DWAF, 1996c) are 0.002 mg/l, 0 to 1.0 mg/l and 0 to 20 mg/l respectively. Compared to water samples, sediments showed maximum concentration of Mn (27.67 mg/l) in station 2. Pb concentration was less both in water and sediment samples. Water showed high Pb content (1.81mg/l) in station 2 and sediment showed high (2.11 mg/l) in station 1. The levels of Pb obtained in sediment were higher than those in the lake water; hence the sediment could be an influential factor on the level of Pb in water with other enhancing factors such as the current flow and pH since water acidity is known to influence the solubility and availability of metals.

Conclusion

The elevated values identified for cadmium, copper and chromium in the study area indicate that the surface sediments are extremely contaminated, probably as a result of anthropogenic activities and provide a useful means of distinguishing between the natural and anthropogenic sources of metal entering in to the coastal zone. Elevated levels of Cd and Pb were detected in the river, which could be directly detrimental to the health of the aquatic ecosystem and indirectly to man. Heavy metals are most considerable contaminants in water and soil.. Excess levels of heavy metals might cause health effects to the human beings. ICP-AES is one of the most widely and universally using technique for determination of heavy metals up to trace levels.

REFERENCE

Ademoroti CMA. 1996. Level of heavy metals on bark and fruit of trees in Benin City, Nigeria. *Environ. Pollut.* (Series B). 11: 241-253.

Adeniyi A.A, Yusuf K.A, Okedeyi O. 2008. Assessment of the exposure of two fish species to metals pollution in the Ogun river catchments, Ketu, Lagos, Nigeria. *Environ. Monit. Assess.,* 137: 451- 458.

Akbal, F., Gurel, L., Bahadyr, T., Guler, I.L, Bakan, G., Ngor, Y.K. 2011. Water and Sediment quality assessment in the mid-black sea cost of Turkey using multivariate statistical techniques. *Environmental earth Science.* 64: 1387-1395.

Akkoyunlu, A.E and Akiner, M. 2012. Pollution evaluation in streams using water quality indices: A case study from Turkey's Sapanca Lake Basin. *Ecological indicators*. 501-511.

APHA, AWWA, WEF. 1995. Standard Methods for Examination of Water and Wastewater, 20th Edition, Volume.1American Public Health Association, Washington,1368.

Ayejuyo, O., Omojole, M.O., Ojoniyi, O.J. 2010. Sediment load and bioconcentration of heavy metals by shrimp (*Peaneus notalis*) from Epe Lagoon, Nigeria. *Environ. Monit. Assess.,* 163: 295-301.

Bai, J., Cui, B., Chen, B., Zhang, K., Deng, W., Gao, H., Xiao, R. 2010. Spatial distribution and ecological risk assessment of heavy metals in surface sediments from a typical plateau lake wetland, *China. Ecol. Modeling*, 222: 301-306.

Binning, K., Baird, D. (2001). Survey of heavy metals in the sediments of the Swart kops River Estuary, Port Elizabeth South Africa. *Water SA.* **27:** 461–466.

Censi, P., Spoto, S. E., Saiano, F., Sprovieri, M., Mazzola, S., Nardone, G., Di Geronimo, S. I., Punturo, R., Ottonello, D. (2006). Heavy metals in coastal water systems. A case study from the northwestern Gulf of Thailand. Chemosphere. 64: 1167–1176.

Dang The Cuong. (2005). Heavy Metal Contamination in Mangrove Habitats of Singapore. Measurement and behaviour of heavy metals in marine environment of Singapore, 102 .

Dara S.S. A Text book of Environmental Chemistry and Pollution control. S. Chand and Company Limited. New Delhi. 2003. 216.

Deng HA, Zhan J, Wang D, Chen Z, Zu C. (2010). Heavy metal pollution and assessment of the tidal flat sediments near the coastal sewage outfalls of Shanghai, China Environ. Earth Sci. 60: 57-63.

Department Of Water Affairs & Forestry, DWAF. (1996b) *Water Quality Guidelines, Aquatic Ecosystem Use*. Vol.7 (1st edn.) DWAF. Pretoria.

Department Of Water Affairs & Forestry, DWAF. (1996c) *Water Quality Guidelines, Agricultural Use and Livestock Watering*. Vol. 5 (2nd edn.) DWAF. Pretoria.

Eddy N.O and Etuk B.A. (2004a). Seasonal variability of physic-chemical parameters of Calabar River estuary: Influence of tide and season. J. Sci. Total Environ. 48: 14-119.

Eddy N.O and Etuk B.A. (2004b). Seasonal variability of physic-chemical parameters of Calabar River estuary: Influence of tide and season. J. Sci. Total Environ. 48: 14-119.

Eddy N.O, Udoh C.L and Ndibuke M.O. (2004). Heavy metals in sediments from Cross River at Oron. Afr. J. Envir. Sci. Health. 7: 115-119.

Egboh S.H.O and Emeshili E.M. (2007). Physico- chemical characteristics of River Ethiope source in Umuaja, Delta State, Nigeria. J. Chem. Soc. Nigeria. 32 (2): 72-76.

Fatoki O.S and Awofolu O.R. (2003) . Levels of Cd, Hg and Zn in some surface waters from the Eastern Cape Province, South Africa. *Water SA*. 29 (4) 375-380.

Fatoki O.S, Lujiza N and Ogunfowokan A.O. (2002) Trace metal pollution in Umtata River. *Water SA* 28 (2) 183-190.

Fernandes, C., Fontaínhas-Fernandes, A., Cabral, D., Salgado, M. A. (2008). Heavy metals in water, sediment and tissues of *Liza saliens* from Esmoriz–Paramos lagoon, Portugal. Environ. Monit. Assess. 136: 267–275.

Halcrow W, Mackay D.W, Thornton I. (2007). The distribution of trace metals and fauna in the Firth of Clyde in relation to the disposal of sewage sludge. J. Mar. Biol. Ass. U.K.: 53: 721-739.

Haque M.I. Water resources management in Bangladesh. Anushilan, Chuadanga and Dhaka, 2008, 28-84.

Islam MS, Suravi, Meghla NT. (2010). Investigation on water quality in the Ashulia beel, Dhaka. Bangladesh J. Fish. Res.14(1-2): 55-64.

Kackstaetter, U. R., Heinrichs, G. (1997). Validity of low cost laboratory geochemistry for environmental applications. Water Air Soil Pollut. 95: 119–131.

MacFarlane, G. B., Burchettt, M. D. (2000). Cellular distribution of Cu, Pb, and Zn in the Grey Mangrove Avicemnia marina (Forsk.). Vierh Aquatic Botanic. 68: 45–59.

Malik N, Biswas A.K, Qureshi T.A, Borona K, Virha R. (2010). Bioaccumulation of heavy metals in fish tissues of a fresh water lake of Bhopal. Environ. Monit. Assess.160: 267.

Martin IM, Whitefield M. (1983). The significance of river input of chemical element to the Ocean In: Wong C.S., Boyle, E., Bruland, K.W. (eds.). Trace metals in sea water. Plenum, New York, pp. 265- 296.

Mohiuddin K.M, Zakir H.M, Otomo K, Sharmin S, Shikazono N. G. (2010). Geochemical distribution of trace metal pollutants in water and sediments of downstream of an urban river. International Journal of Environmental Science and Technology.7:17-28.

P. C. Prabu. (2009). "Impact of heavy metal contamination of Akaki river of Ethiopia on soil and metal toxicity on cultivated vegetable crops," *Electronic Journal of Environmental, Agricultural and Food Chemistry*. vol. 8 (9); 818–827.

Rahman AKML, Islam M, Hossain M.Z, Ashan M.A. (2012). Study of the Seasonal variations in Turag river water quality parameters. Afr. J. Pure and Appl. Chem. 6 (10): 144-148.

Rosli N.A, Zawawi M.H, Bustami R.A. (2012). Salak river water quality identification and classification according to physico – chemical characteristics. International Conference on Advance science and Contemporary Engineering. 69-77.

Shikazono N, Tatewaki K, Mohiuddin K.M, Nakano T, Zakir H.M. (2012). Sources, spatial variation and speciation of heavy metals.

Stoeppler M. (1991). Cadmium. In: Merian E (ed.) *Metals and their Compounds in the Environment*: *Occurrence, Analysis and Biological Relevance*. VCH, New York. 803-851.

Tukura.B.W., Gimba C.E.,Ndukwe I.G.,Kim B.C. (2012). Physicochemical characteristics of water and sediments in Mada river, Nasarawa state, Nigeria, International journal of environment and bioenergy. 1(3): 170-178.

US EPA. (2002). SW-846 reference methodology: Method 3050B. Standard Operating Procedure for the Digestion of Soil/Sediment Samples Using a Hotplate/Beaker Digestion Technique, Chicago, Illinois.

Usero J, Morrilo J, Graccia I. (2005). Heavy metal concentrations in molluscs from the Atlantic coast of Southern Spain. Chemosphere, 59: 1175-1181.

Verardo, D.J., Froelich, P.N., McIntyre, A.(1990). Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 analyzer. Deep Sea Research 37, 157–165.

Washington D. C "Assessment of heavy metals in some dumps of copper mining and plants in Mirdita Area, Albania," in *Proceedings of the 5th International Scientific Conference on Water, Climate and Environment*, S. Kane, P. Lazo, and A. Flora, Eds., Ohrid, Macedonia, (2012).

World Health Organization (WHO), Drinking Water Sanitation. A Way to World Health Organization. Geneva.1993.

World Health Organization (WHO,2008), Guidelines for Drinking-water Quality, 3rd Edition.

WPCL (Water Pollution Control Legislation, 2004). Land-Based Water Quality Classification, *Official Journal,* 25687, Turkey.

Yusuf K.A, Osibanjo O. (2006). Trace metals in water from Ologe Lagoon, Lagos, Nigeria. ASSET., 3: 121-128.

Yusuf KA, Osibanjo O. (2006). Trace metals in water and sediments from Ologe lagoon, southwestern Nigeria. Pak. J. Sci. Ind. Res., 49: 88-89

Zakir H.M, Kohinoor Begum, Mohiuddin K.M, Arafat M.Y. (2011). Quality assessment of waters of Borga city area, Bangladesh. Journal of Agro forestry and Environment 2011; 5: 21-25.

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